MICRO 7803

DATA QUALITY ASSURANCE IN RECREATIONAL LAKES MOBILE LABORATORIES 1975-1977 REPRODUCIBILITY OF ANALYSES

GEORGE HENDRY, ALLAN BURGER AND WENDY MOSS

MICROBIOLOGY REPORT

LABORATORY SERVICES BRANCH

ONTARIO MINISTRY OF THE ENVIRONMENT

SEPTEMBER 1978

MOE
DAT
ANUA

c.1
a aa

Copyright Provisions and Restrictions on Copying:

This Ontario Ministry of the Environment work is protected by Crown copyright (unless otherwise indicated), which is held by the Queen's Printer for Ontario. It may be reproduced for non-commercial purposes if credit is given and Crown copyright is acknowledged.

It may not be reproduced, in all or in part, for any commercial purpose except under a licence from the Queen's Printer for Ontario.

For information on reproducing Government of Ontario works, please contact ServiceOntario Publications at copyright@ontario.ca

SUMMARY

Approximately one in twenty of Lakeshore Capacity and recreational lakes samples from three mobile laboratories were duplicated. The index of dispersion (D^2) was calculated for each pair of results. The data were also examined by regression analysis. The repoducibility was determined for the analysis of each water quality parameter, and a comparison of reproducibility of data from the three laboratories was made. Evidence was obtained to show that in some cases high background counts interfere witn the total coliform or fecal streptococcus analyses. Poor reproducibility was obtained for some experimental methods used for the Lakeshore Capacity program. Analyses of the standard parameters, total coliform, fecal coliform, and fecal streptococcus, reproduced equally well in all three mobile laboratories.

anua

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Every variable concerning the operation of a laboratory which influences the microbiology methods, and hence the laboratory's results, must be monitored by the quality control program. A program to control the purchase, preparation and storage of media, as well as selection of equipment, has recently been established(9). Certain aspects of data quality assurance were also investigated recently(10).

A data quality assurance program for Recreational Lakes Mobile laboratories was implemented in 1975. It consisted of a measure of reproducibility of bacterial density measurements, and some identification of isolates of the water quality parameters. The program was small enough in scope not to interfere greatly with regular work, yet it did provide some assurance over a long period that the laboratories were functioning satisfactorily.

The data quality assurance program served at least three purposes:-

- It monitored the reproducibility of the data and allowed for the detection of errors in techniques.
- It encouraged technicians to be careful because they were informed that their work could be checked on a routine basis.
- The quality assurance measurements were used to assure those who used our services that the mobile laboratory operations were sound.

The technicians knowledge that a particular sample is a replicate analysis makes them understandably more careful in their treatment of the sample.

This may result in a loss of some variability between replicates. A sample for replication always contains much less volume of water than did the original sample so that relabelling the bottle does not adequately disguise the replicate. It is possible to split a large sample into sub-samples, and submit them disguised as samples from an outside source, but this must be handled by or accomplished with personnel other than those being tested. If one cannot take these precautions, then one is left with data containing less than the full variability characteristic of the laboratory under investigation. Such data can be used by preparing a control chart from which new control levels can be devised. An illustration was given by Eisenhart and Wilson(3).

The purposes of this Quality Assurance report is to document the results of the replication of analyses and to decide if there are differences in performance among the various mobile laboratories. A second report will deal with the identification of coliforms isolated from recreational lakes.

METHODS

Bacterial Enumeration

1) Total coliform (TC) bacteria were determined as a count of dark red colonies with gold metallic sheen grown on a membrane filter (Gelman GN6) with m-Endo LES agar(4).
2) Fecal coliform (FC) bacteria were determined by counting yellow to yellowish-green colonies grown on a membrane filter with MacConkey broth at 44.5^{0}C.(4).

3) Fecal streptococcus (FS) bacteria were obtained from a count of pink or red colonies grown on a membrane filter with m-Enterococcus agar(4).
4) Pseudomonas aeruginosa (PA) was determined as a count of flat, tan to brown, colonies grown on a membrane filter with mPA Agar(5).
5) Heterotrophic bacteria (HB) were obtained from a total count of colonies from surface inoculated plates of modified Foot and Taylor medium, incubated for seven days at 20°C.(7).
6) Aeromonas was determined as a count of yellow colonies grown on a membrane with Rimler-Schott's medium(6).
7) Acinetobacter was determined as a count of blue colonies grown on a membrane with EMB agar (pH 10.0) (11).

Index of Dispersion

Approximately one in twenty of the routine determinations was duplicated by the mobile laboratory operator. In the Dorset laboratory when the mobile laboratory operator performed the routine analysis, another technician, or very rarely a summer student carried out the duplicate analysis. When the routine analyses were completed, samples were randomly selected for quality control testing. The original and duplicate analyses were taken from the same sample, but as the sample remaining after the regular analysis was small, each parameter required different samples.

The data pairs were tabulated, and the results deleted when either original or duplicate counts was zero. As index of dispersion (D^2) was calculated for each pair of original and duplicate counts. The formula for D^2 using a pair of variables X_1 and X_2 is:

$$D^2 = \frac{(X_1 - X_2)^2}{X_1 + X_2} = \frac{\Sigma (x - \bar{x})^2}{\bar{x}}$$

A discussion of D^2 and its applications to microbiological testing has been published(1,3). Unacceptable D^2 values were considered to be those which exceeded the 95% confidence limits, and were marked by an asterisk. A tally was made of the D^2 values for each parameter and laboratory. The percentage of unacceptable values was used as a measure of the reproducibility of the data. The variability within the data from each source is suggested to be unacceptably high when the fraction of those D^2 values exceeding the 95% confidence limits is greater than 15% of at least 20 environmental samples examined (1).

REGRESSION ANALYSIS

The data from three laboratories were modified by a square root transformation. A linear regression of the data was then plotted for each parameter and laboratory, where possible compared by analysis of covariance. The original values were plotted on the X-axis and the duplicate value on the Y-axis. (Figures 1-7). Several statistical analyses were conducted. The degrees of freedom (df), slope, and intercept were determined for each line; a t-test performed on the slope and intercept revealed significant differences from a slope of 1, or a zero intercept respectively. The lines were compared using the t-test and Bartlett's approximate Chi-square test calculated on the sum of squares within each set of data.

RESULTS

The frequency of high D^2 values from the two mobile laboratories were compared (Table 1). A comparison was made

TABLE 1

Comparison of D^2 Values for Two Mobile Laboratories.

Laboratory Parameter		Frequency of D^2 Values				
		Acceptable†	Not Acceptable	Total	% Not Acceptable	X^2
#5	TC	41	8	49	16.3	0.000
#6		36	7	43	16.3	
TOTAL		77	15	92	16.3	
#5	FC	22	2	24	8.3	0.037
#6		18	2	20	10.0	
TOTAL		40	4	44	9.1	
#5	FS	20	0	20	0	3.987*
#6		23	5	28	17.6	
TOTAL		43	5	48	10.4	

* X^2 significant at $P \leq 0.05$ level

† An acceptable D^2 value can be obtained by chance alone.

of D^2 values obtained from the mobile laboratories and the Dorset laboratory (Table 2). The D^2 values for the analysis of heterotrophic bacteria, Aeromonas, and Acinetobacter obtained from the Dorset laboratory are shown in Table 3. The results of the regression analysis of each parameter, and the comparison of results from different laboratories, are illustrated in figures 1,2,3,4,5,6, and 7.

Discussion

Index of Dispersion

A Comparison of the Reproducibility of Each Water Quality Parameter

The Chi-Square Test confirmed that the results of duplication of fecal streptococci analyses from laboratories 5 and 6 were significantly different. The reason for the poorer performance of laboratory six with this analysis was likely some unusual characteristic of the sample. One such characteristic was the high level of FS background colonies. Four of five of the unacceptably high D^2 values from laboratory 6 were produced from samples taken from Mississippi lake which was the only lake to yield noticeable numbers of FS background colonies. Forty percent of colonies on enterococcus agar plates (fecal streptococci counts) from Mississippi lake were red pin-point colonies which were identified as Lactobacillus SP.(8). The Lactobacillus colonies were background to the target colonies (Streptococci) and their numbers were high enough to have cause some interference with the reproducibility of counting the fecal streptococcus colonies. The difference in reproducibility of fecal streptococcus is illustrated in figure 8. The use of this type of performance chart has been

TABLE 2

Comparison of D^2 Values for Two Mobile Laboratories With The Dorset Laboratory

Laboratory	Parameter	Frequency of D^2 Values: Acceptable†	Not Acceptable	Total	% Not Acceptable	X^2
Mobile	TC	77	15	92	16.3	0.967
Dorset	TC	22	2	24	8.3	
TOTAL	TC	99	17	116	14.7	
Mobile	FC	40	4	44	9.1	0.025
Dorset	FC	12	1	13	7.7	
TOTAL	FC	52	5	57	8.8	

* X^2 significant at $P \leq 0.05$

† An Acceptable D^2 value can be obtained by chance alone.

TABLE 3

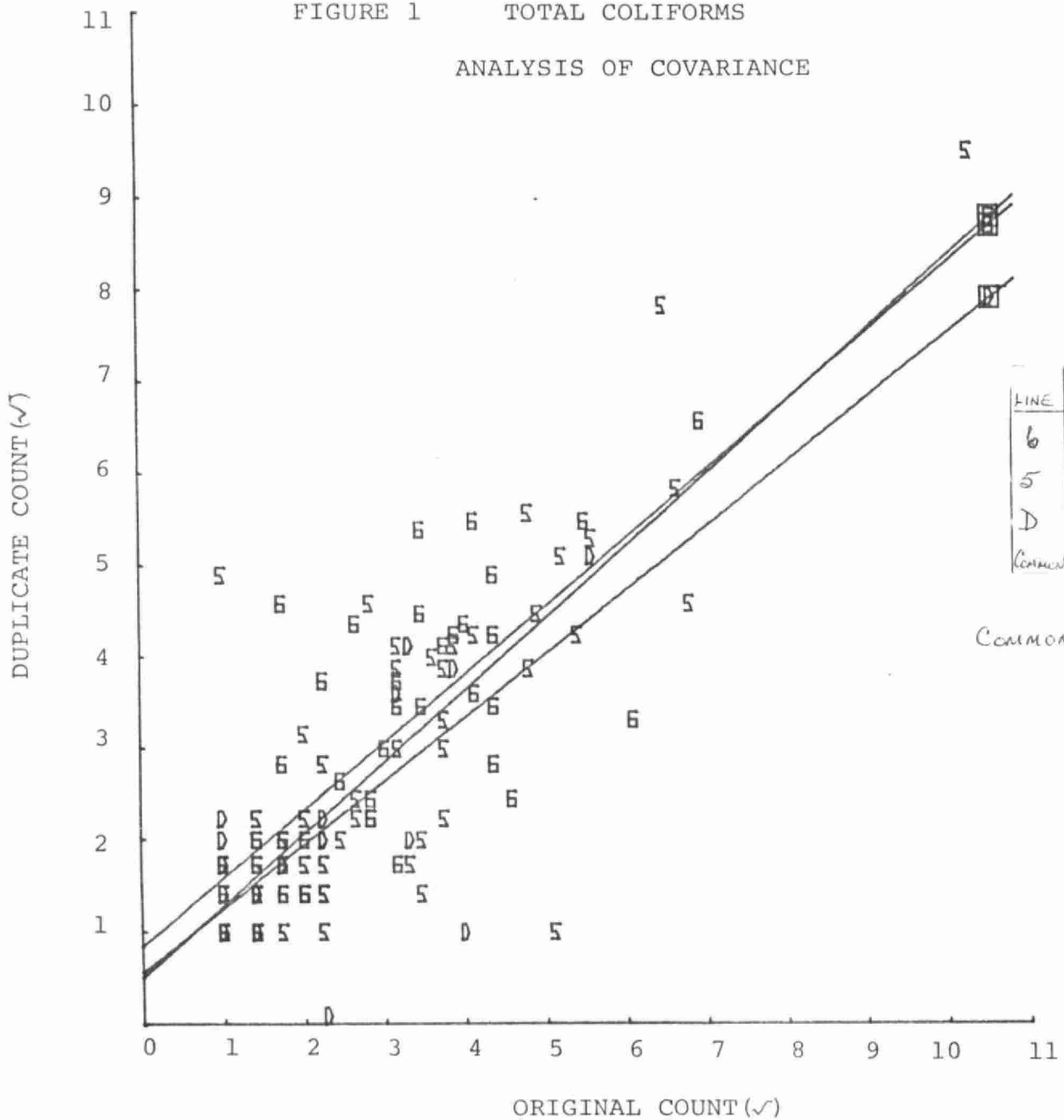
D^2 Values For The Dorset Laboratory

Parameter	Frequency of D^2 Values			
	Acceptable†	Not Acceptable	Total	% Not Acceptable
Heterotrophic Bacteria	14	9	23	39.1
Aeromonas	25	10	35	28.6
Acinetobacter	11	3	14	21.4

† An Acceptable D^2 Value can be obtained by chance alone.

FIGURE 1 TOTAL COLIFORMS

ANALYSIS OF COVARIANCE



KEY

5 - MOBILE LABORATORY #5

6 - MOBILE LABORATORY #6

D - DORSET LABORATORY

LINE	df	SLOPE	t VALUE	INTERCEPT	t VALUE	TABLE t VALUE
6	41	0.7414	2.513	0.5484	2.548	2.021
5	47	0.7526	2.528	0.5094	1.591	2.021
D	22	0.6938	1.991	0.2689	1.50	2.019
Common	112	0.7595	4.015	0.6300	3.20	1.980

COMMON SLOPE REG COEF F VALUE .05 - 0.130

TABLE F VALUE .05 - 3.07

ADJ MEAN F VALUE .05 - 1.099

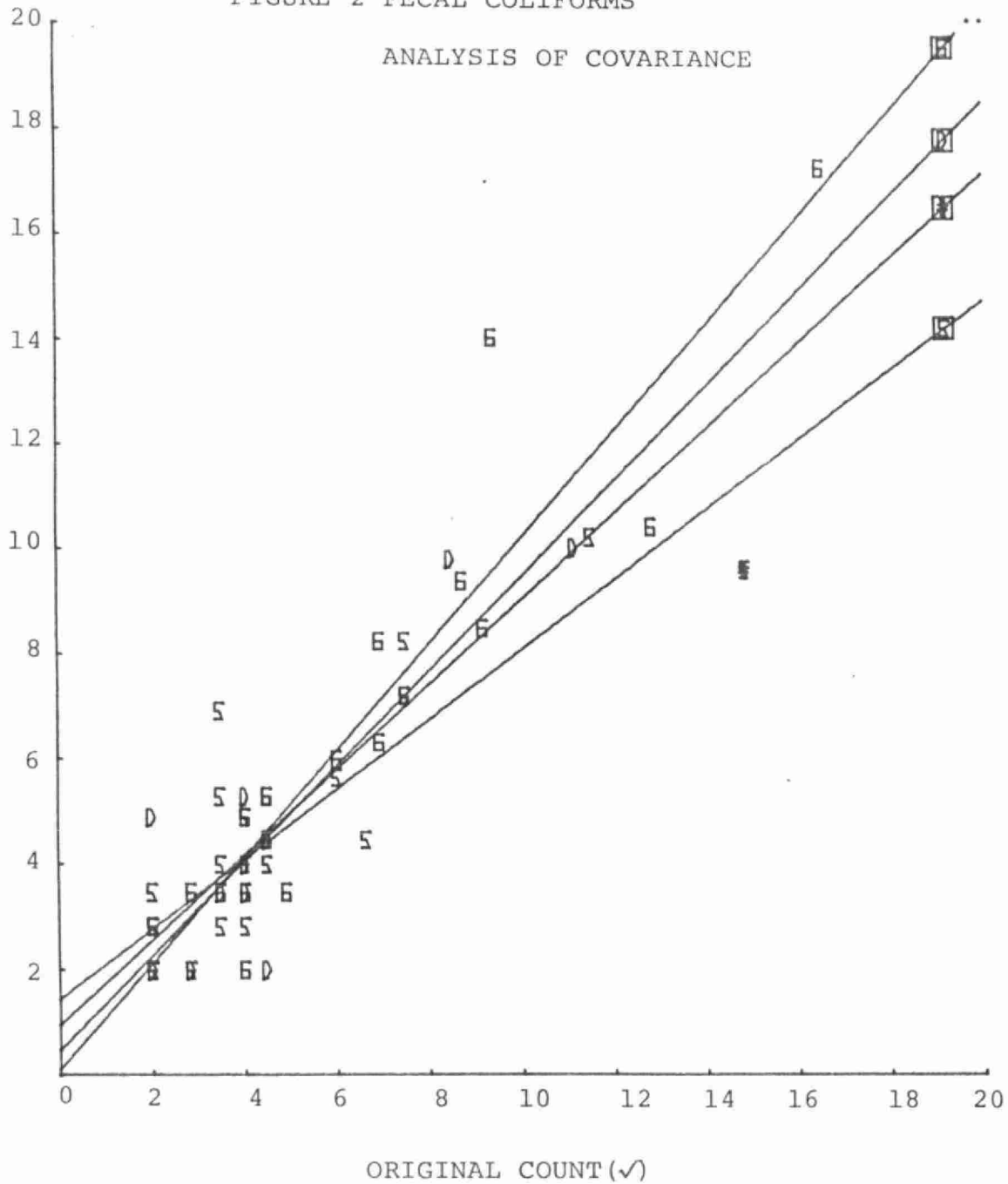
TABLE F VALUE .05 - 3.07

BARTLETT'S CHI SQUARE .05 - 1.61

TABLE CHI SQUARE .05 - 5.991

FIGURE 2 FECAL COLIFORMS

ANALYSIS OF COVARIANCE



KEY

5 - MOBILE LABORATORY #5

C - MOBILE LABORATORY #5 MINUS 1 POINT

6 - MOBILE LABORATORY #6

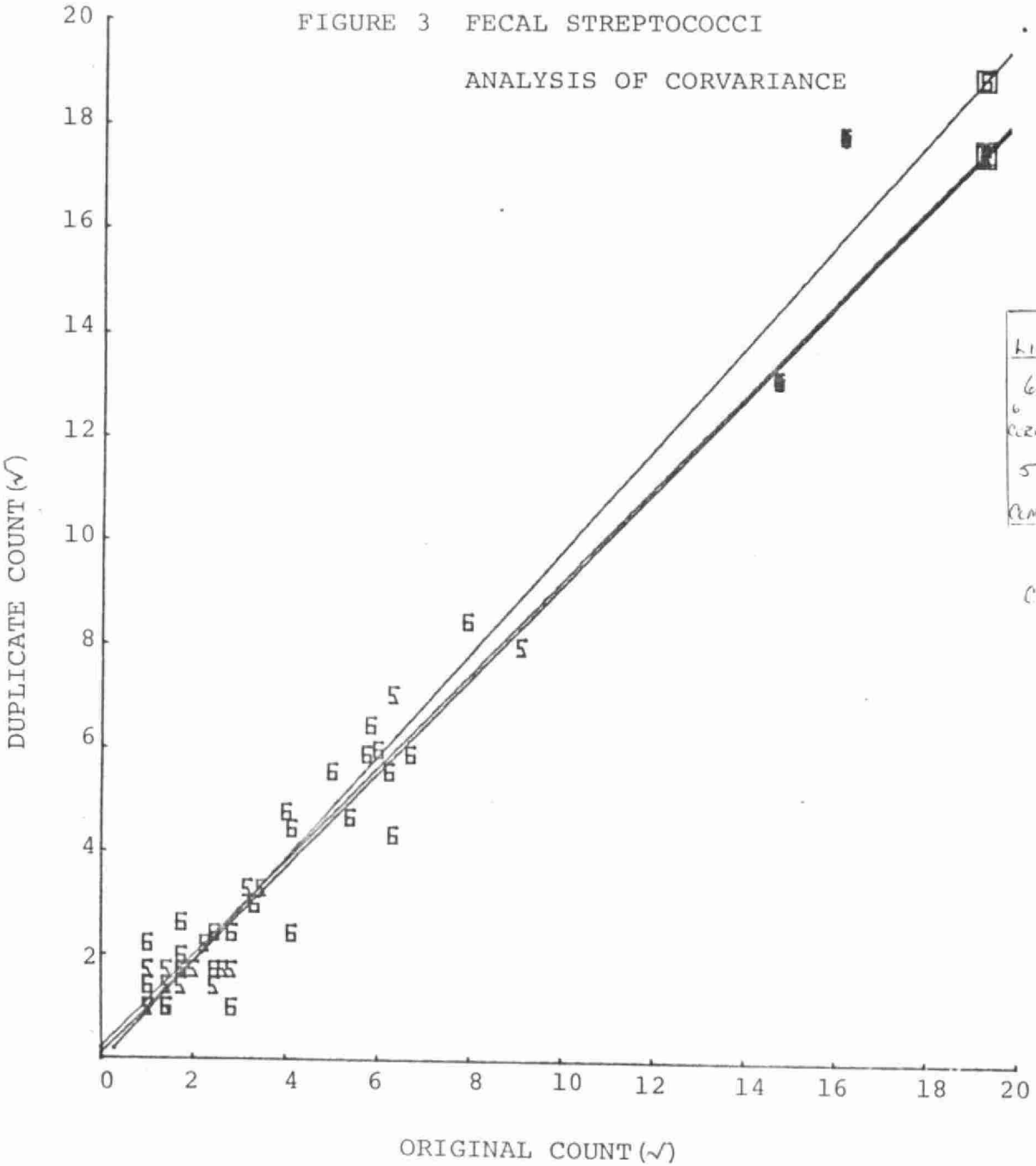
D - DORSET LABORATORY

LINE	df	SLOPE	t VALUE	INTERCEPT	t VALUE	TABLE t VALUE
6	17	1.0057	0.088	0.0606	0.166	2.101
5	21	0.6615	4.329	0.7372	3.417	2.074
C CORRECTED	20	0.8070	-1.942	0.4839	2.084	2.050
D	10	0.5957	-0.665	0.2452	0.628	2.201
COMMON	49	0.9314	0.690	0.2476	1.360	2.010

COMMON SLOPE	REG COEF. F VALUE	.05	0.969
	TABLE F VALUE	.05	4.08
	ADJ MEAN F VALUE	.05	0.147
	TABLE F VALUE	.05	4.08
	BARTLETT'S CHI SQUARE	.05	1.80
	TABLE CHI SQUARE	.05	5.991

FIGURE 3 FECAL STREPTOCOCCI

ANALYSIS OF CORVARIANCE



KEY

5 - MOBILE LABORATORY #5

6 - MOBILE LABORATORY #6

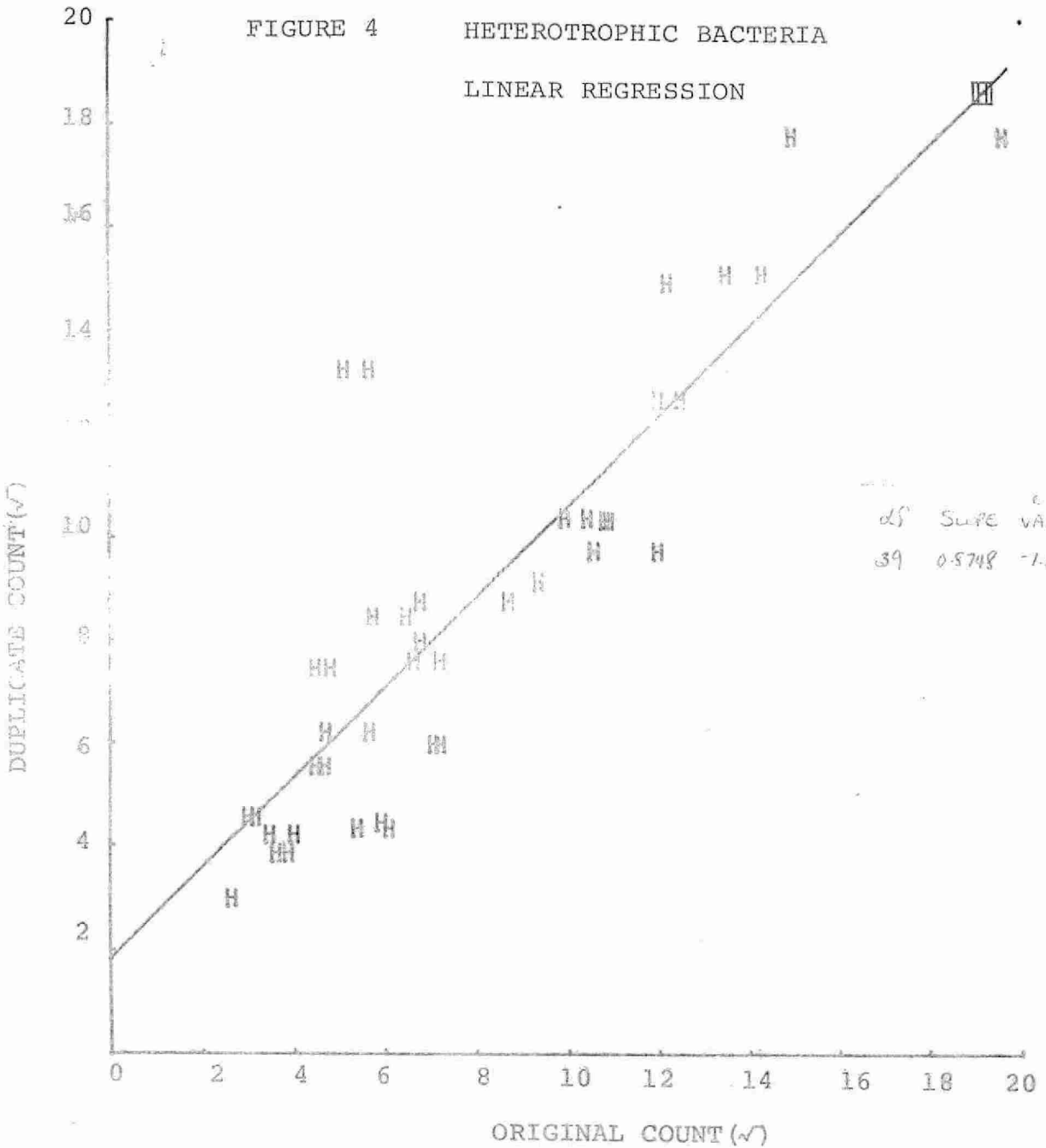
C - MOBILE LABORATORY #6 MINUS 2 POINTS

LINE	df	SLOPE	t VALUE	INTERCEPT	t VALUE	TABLE t VALUE
6	25	0.9742	0.117	-0.0797	0.2748	2.036
6 CORRECTED	23	0.9067	-1.163	0.2204	0.6476	2.064
5	18	0.9105	-1.476	0.0867	0.4394	2.093
COMMON	42	0.9083	-1.724	0.1601	0.8031	2.021

COMMON SLOPE	REG COEF. F VALUE	.05	0.001
	TABLE F VALUE	.05	4.08
	ADJ MEAN F VALUE	.05	0.299
	TABLE F VALUE	.05	4.08
	BARTLETT'S CHI SQUARE	.05	3.04
	TABLE CHI SQUARE	.05	3.841

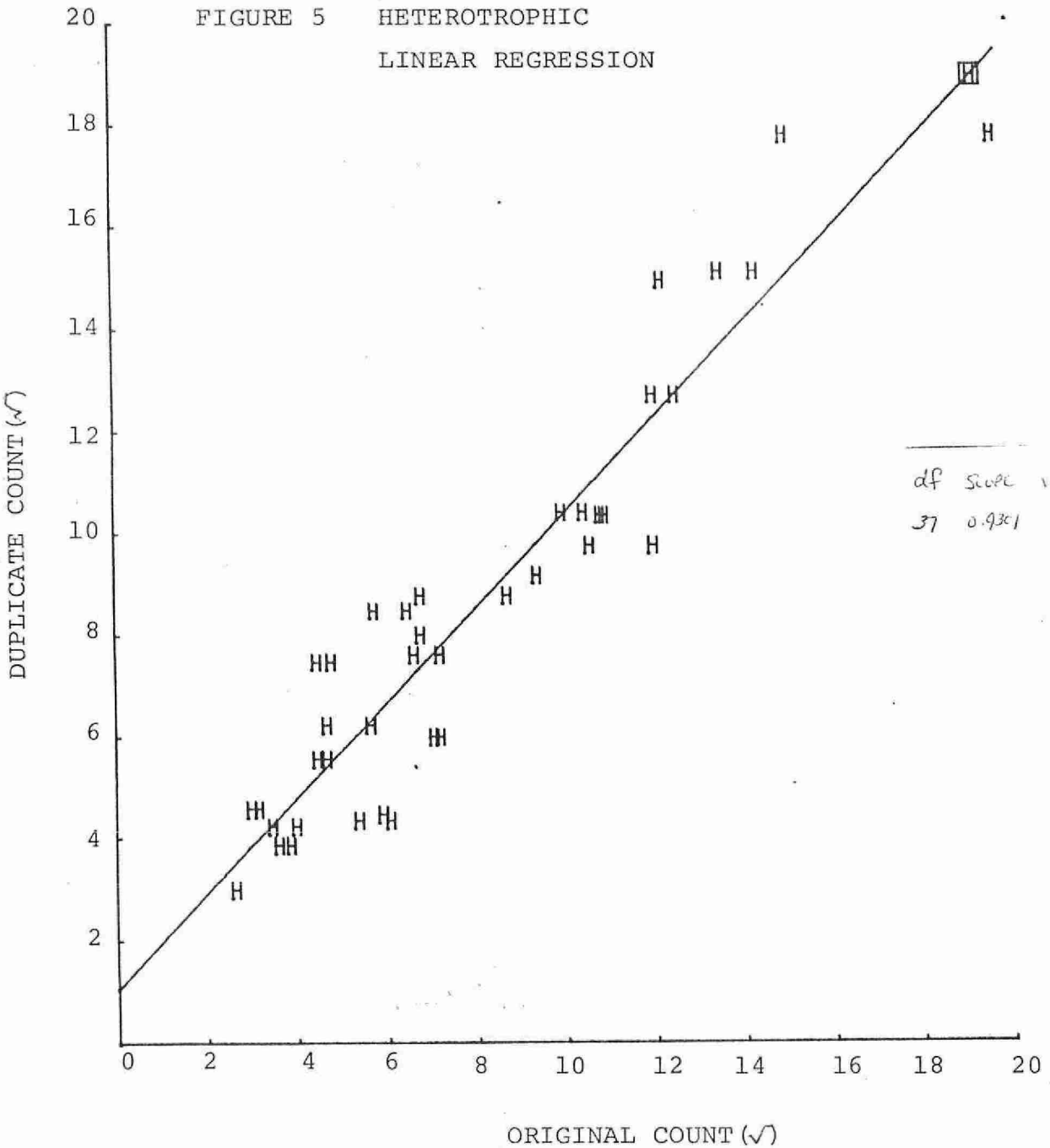
FIGURE 4 HETEROTROPHIC BACTERIA

LINEAR REGRESSION



df	Slope	t value	Intercept	F value	t value
39	0.5748	1.503	1.8500	2.573	2.021

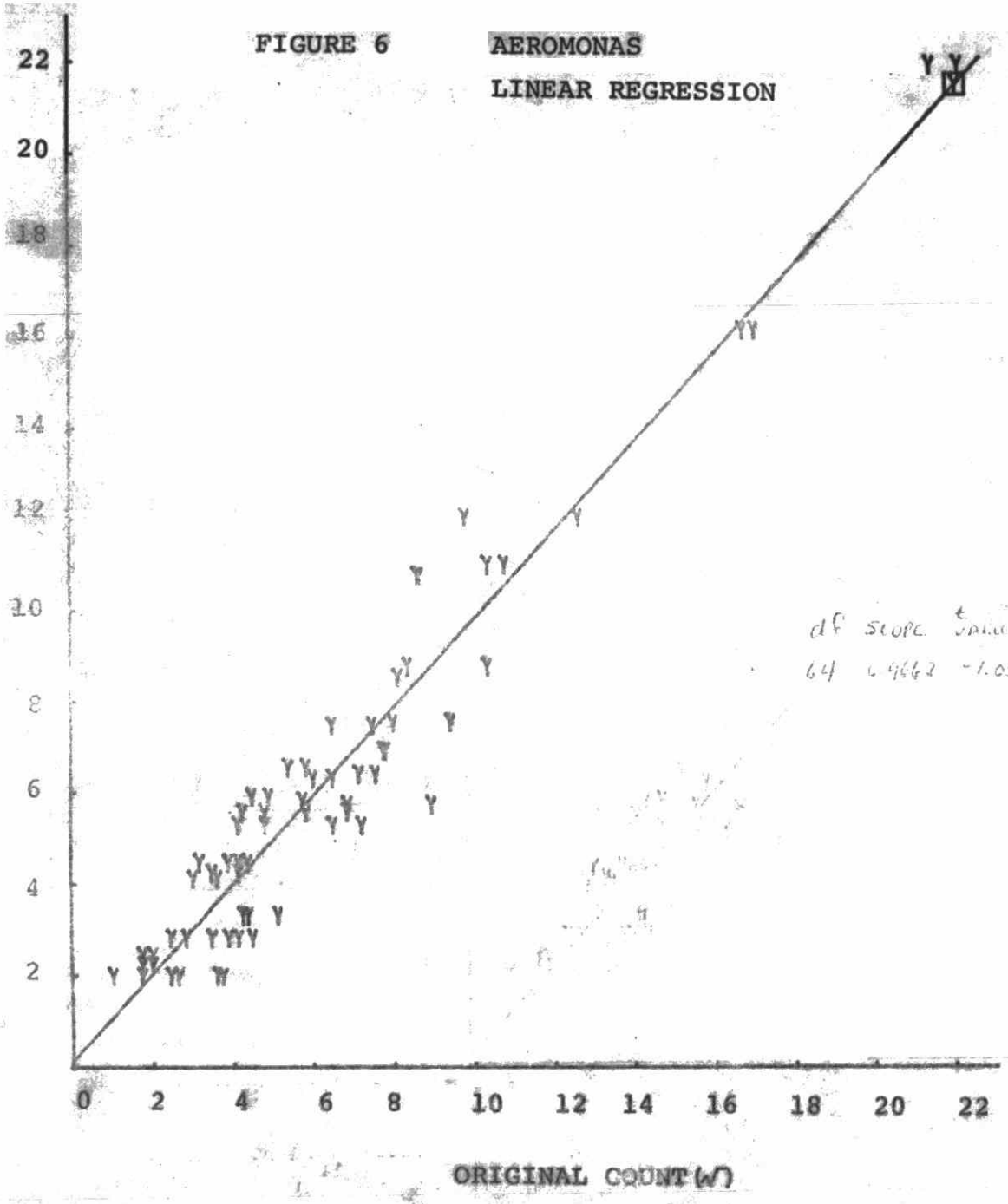
FIGURE 5 HETEROTROPHIC
LINEAR REGRESSION



df	SLOPE	t VALUE	INTERCEPT	t VALUE	TABLE t VALUE
37	0.931	1.255	1.0719	2.198	2.021

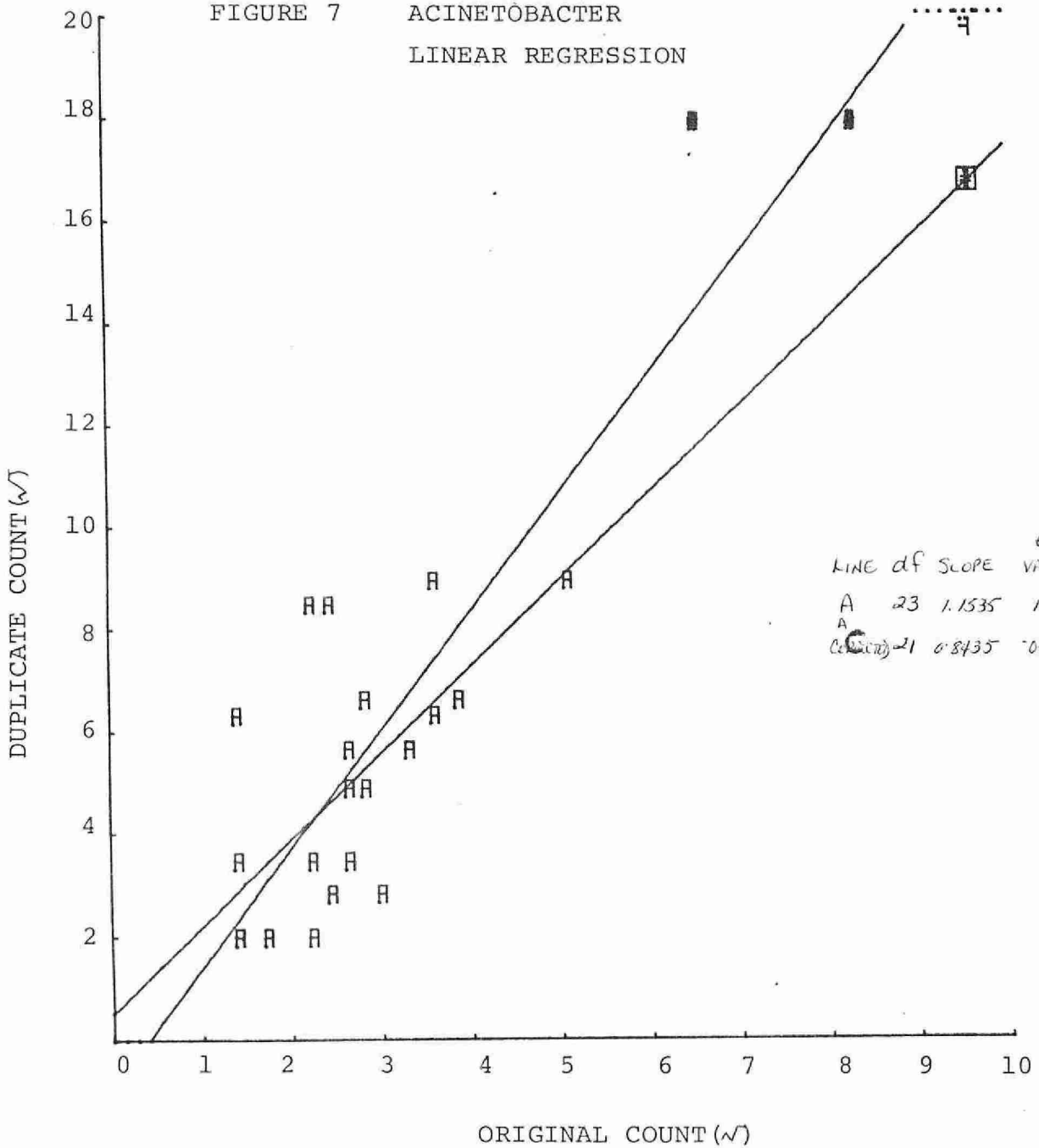
FIGURE 6 AEROMONAS

LINEAR REGRESSION



df	SLOPE	t VALUE	INTERCEPT	t VALUE	TABLE t VALUE
64	0.9662	-1.056	0.1455	0.6048	3.000

FIGURE 7 ACINETOBACTER
LINEAR REGRESSION



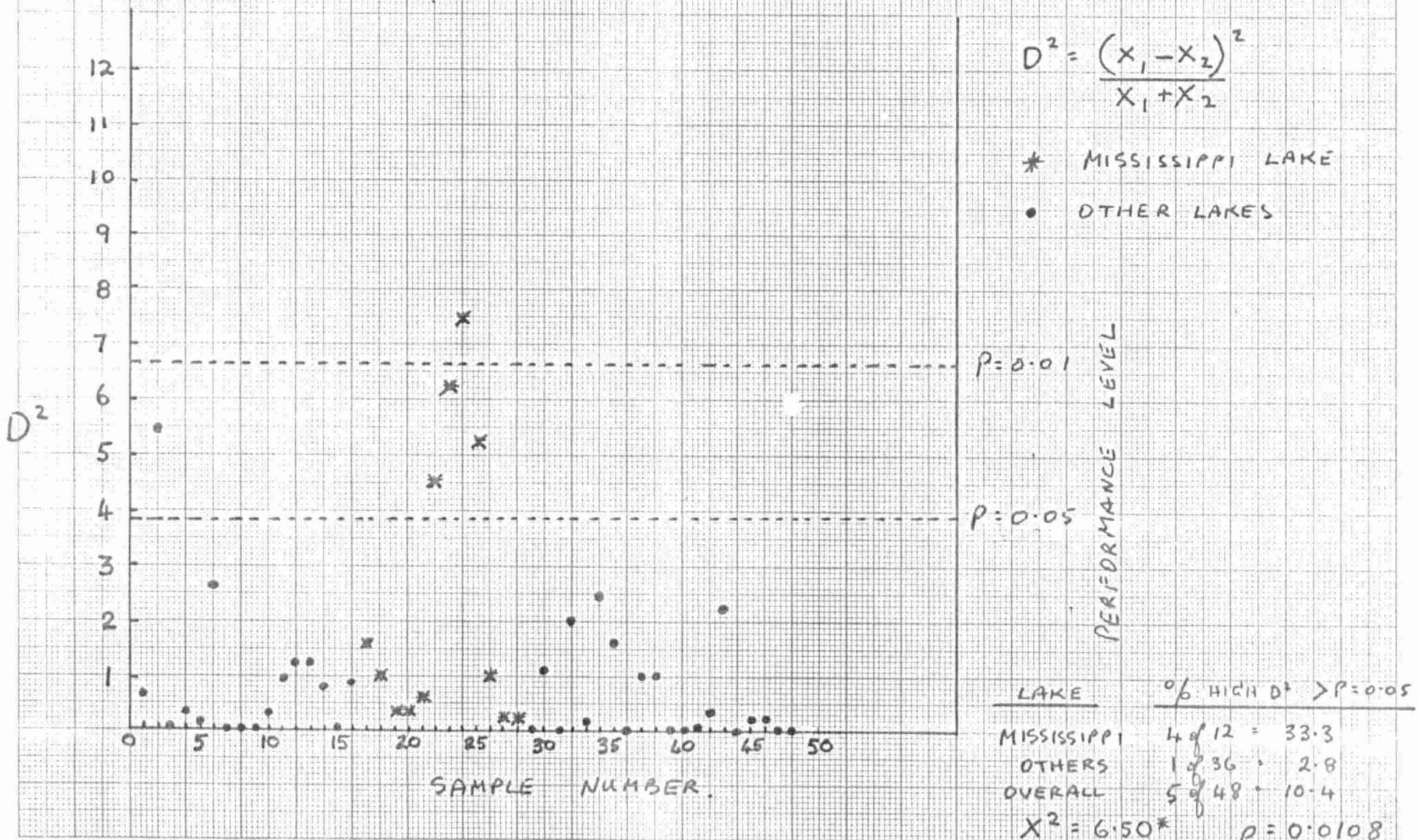
KEY

A - ACINETOBACTER

C - ACINETOBACTER MINUS 2 POINTS

LINE	df	SLOPE	t VALUE	INTERCEPT	t VALUE	TABLE t VALUE
A	23	1.1535	1.204	0.4440	-1.695	2.064
C	21	0.8435	0.733	0.2657	0.463	2.074

FIGURE 8. PERFORMANCE CHART - INDEX OF DISPERSION (D^2)
FOR DUPLICATED FECAL STREPTOCOCCUS ANALYSES



LAKE	% HIGH D^2 > P = 0·05
MISSISSIPPI	4 of 12 = 33·3
OTHERS	1 of 36 = 2·8
OVERALL	5 of 48 = 10·4

$X^2 = 6{\cdot}50^*$ $\quad P = 0{\cdot}0108$

suggested before (3), and has occasional use in the more modern literature (eg.2).

Interference by background colonies was not restricted to the fecal streptococcus analysis. Total coliform background colonies have been shown to interfere with total coliform formation (7), and this may be the cause of the high D^2 values from TC analysis reported from both mobile laboratories in this study. The level of unacceptable D^2 values for Total Coliform determinations was 16%, while that for the Fecal Coliform analysis was within the acceptable range. The reproducibility of both the Total Coliform and Fecal Coliform data produced in each of the two mobile laboratories was good (Table 1). However the total coliform analysis was less reproducible than the fecal coliform analysis which had few background colonies when recreational lake samples were used. River and sewage samples have much higher background counts and the effect this may have on the D^2 values is presently being investigated.

A limited amount of total coliform data was available where two different volumes of water were used from the same sample. An association was noted between an increase in D^2 value with increase in sample volume only in those samples which gave an unacceptable D^2 value with the lower sample volume (Table 4). D^2 is independent of sample volume. Therefore the unacceptable D^2 values are linked in a nonrandom manner to specific samples. The special characteristic of these samples may be the level of background colonies. This problem deserves to be investigated more fully.

TABLE 4

Association of An Increase in D^2 value with Increase in Sample Volume in Unacceptable Samples Only.

		Level of D^2 Value For The Low Sample Volume		
		Acceptable	Unacceptable	Total
Change In D^2 With Increase In Sample Volume	Decrease	5	0	5
	Increase	2*	3	5
Totals		7	3	10

$X^2 = 4.286$ $P = 0.00384$

† An acceptable D^2 value can be obtained by chance alone

* Although there was an increase in D^2 values they remained in the acceptable range.

A Comparison of Reproducibility From Three Laboratories

The D^2 values from the two mobile laboratories and the Dorset labatory were compared (Table 2). There were no significant differences between the three laboratories with respect to the reproducibility of the data from the Total Coliform and Fecal Coliform results.

Lakeshore Capacity Data From The Dorset Laboratory.

The levels of unacceptable D^2 values for Total Coliform and Fecal Coliform produced in the Dorset laboratory were 8.3% and 7.7% respectively which were much below the 15% level of acceptability.

The D^2 values were calculated for counts of Heterotrophic Bacteria, Aeromonas and Acinetobacter carried out for Lakeshore Capacity at the Dorset laboratory. The unacceptable high D^2 values totalled more than 15%, and it was concluded that the reproducibility of these counts was poor (Table 3). The technical problems which led to the production of this unsatisfactory data should be determined, and attempts should be made to correct them. The methods for Aeromonas and Acinetobacter are still at the development stage. As the procedure is improved the D^2 values are likely to decrease. The quality assurace program should be continued so that D^2 values can be compared as the methods go through various stages of improvement.

Regression Analysis

Total Coliforms

A comparison of the total coliform data from the three laboratories is shown in Figure 1. While the slopes of line #5 and #6 vary significantly from 1, the slope of line D (Dorset laboratory) does not, but this was due to the larger error about the line which decreased the sensitivity of the t-test. The slopes of the three lines were not significantly different from one another as judged by the F-test. This analysis confirmed that there were no real differences in reproducibility of data among the three laboratories.

The slopes of the regression lines were all less than one which indicated that on the average the original count was higher than the duplicate. A reduction in bacterial density must have occurred in the waiting period between the original and duplicate analysis. This time period may have been in excess of one hour since it was customary to leave all the duplicate analyses until the end of the day. The results indicated that the time of analysis of duplicates should be as close as possible to that of the original.

Line 6 intercepted the Y-axis at a point significantly different from zero. The intercept value does not necessarily have any quality assurance significance, though a large intercept value can indicate problems with the distribution of data, and these may be corrected. No special data problems could be identified on this occasion.

Fecal Coliforms

The fecal coliform determinations from the three laboratories were compared and the analysis of covariance illustrated in Figure 2. Line 5 had a significant intercept value. The line was redrawn, excluding a pair of atypical data, and resulted in a more satisfactory regression line (Line C), with an intercept value not significantly different from zero. There were no significant differences among the slopes of the three lines, which indicated that the data from the three labs were similar. The slopes of the three lines did not differ significantly from one another which suggested the sample storage problem which resulted in the the death of a small number of Total Coliforms had no significant effect on fecal coliform density.

Fecal Streptococcus

Fecal streptococcus data is illustrated in Figure 3. An intercept problem was found with the data from laboratory 6. Two points were removed which corrected the problem giving line C for laboratory 6. The remaining data were homogenous and none of the laboratories showed differences with any other. The two points removed from laboratory 6 data were of Mississippi Lake data. As mentioned earlier this data had given atypically high D^2 values. There was a tendency for the duplicate count to be lower than the original, however this difference was not statistically significant.

Heterotrophic Bacteria

The original data for heterotrophic bacteria are illustrated in Figure 4. Two atypical points were removed to give the

regression line in figure 5 where the slope and intercept were not different from one and zero respectively.

Aeromonas

The data for replication of Aeromonas are illustrated in Figure 6. The slope and intercept were not significantly different from one and zero respectively. There was a tendency for duplicate counts of heterotrophic bacteria and Aeromonas to be higher than the original count. Neither difference was statistically significant.

Acinetobacter

The data for Acinetobacter are illustrated in Figure 7. The original data, represented by line A, had a slope greater than one, though this was not statistically significant. This slope was possibly in error since all other bacteria had given regression lines with slopes less than one. The slope was made less than one by removing two data points which then gave line C. Both lines A and C gave slopes and intercept values which were not significantly different from one and zero respectively. It is possible that the original data were correct and that Acinetobacter counts were higher in the duplicate than the original. This situation can only be understood through the analysis of more data.

Recommendations and Conclusions.

1) Larger sample bottles were required so that larger volumes of sample could be used. This resulted in fewer data pairs being excluded from calculations because either original or duplicate was zero. The purchase of 500 ml plastic sample bottles in 1977 corrected this problem.

2) The time between original and duplicate analysis should be minimized to reduce the magnitude of changes in bacterial densities. The differences in densities noted in this study were either not statistically significant or in the case of total coliforms averaged only 1 TC/100 ml. Although the changes in density were small they could be measured and were significant. The analysis of original and duplicate within one hour of one another would be a suitable corrective measure.

3) There were no significant differences in reproducibility of data between any of the mobile laboratories.

4) The reproducibility of Heterotrophic bacteria analysis was poor and procedures should be reviewed. Data collection and analysis should be continued as there is no other source of data to which one may refer for guidance in this matter.

5) D^2 values for the experimental methods Aeromonas and Acinetobacter should be collected and compared as the methods go through stages of improvement. A demonstration of this sort is not available in the literature.

6) D^2 values became unacceptably high when analysis of total coliform and fecal streptococcus were conducted on samples containing high densities of background bacteria.

REFERENCES

1) American Society for the Testing of Materials (ASTM). 1976. Interim Recommendations of Committee D-19.

2) Brodsky, M.H., and B.W. Ciebin. 1978. Improved Medium for Recovery and Enumeration of Pseudomonas aeruginosa from Water Using Membrane Filters. Appl. and Environ. Microbiol 36: 36-42

3) Eisenhart, D., and P.W. Wilson, (1943) Statistical Methods and Control in Bacteriology. BACT. REV. 7:57-137.

4) The Enumeration of Pollution Indicator Bacteria, MOE 1976. 24p.

5) Levin, M.A., and Cabelli, V.J., 1972. Membrane Filter Technique for Enumeration of Pseudomonas aeruginosa. Appl. Micro. 24, 6, 864-870.

6) Fliermans, C.B., R.W., Gorden, T.C., Hazen, and G.W. Esch. 1977. Aeromonas Distribution and Survival in a Thermally Altered Lake. Appl. Micro. 33:114-122.

7) Hendry, G.S., 1977. Relationships Between Bacterial Levels and Other Characteristics of Recreational Lakes in the District of Muskoka. Part 1. - Aerobic Heterotrophic Bacteria. Part 2 - Total Coliform Bacteria. MOE 63p.

8) Hendry, G.S., and A. Toth. Mississippi Lake Stury Report. 1977. Mississippi Valley Conservation Authority. 75p.

9) Pagel, Jane E., (1976) Microbiology Quality Assurance Programs. MOE. 43p.

10) Pagel, Jane E., and Bob Hart. 1977. Experience '77 Student Project in Quality Assurance. MOE. 59p.

11) Seyfried, P.L., 1976. Assessment of the Use of Bacteriological Determinants to Study the Effects of Recreational Activity, Development on Lake Water Quality. MOE. 1976. 136p.

	DATE DUE		

MOE/DAT/ANUA
Hendry, G S
Data quality
assurance in recreational lakes anua
mobile laboratories 1975-1977
reproducibility of analysis. c.1 a aa

(9435)
MOE/DAT/ANUA